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Request for grant of a patent

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1.	Your reference	PHM 99-010
2.	Patent application number *(The Patent Office will fill in this part)*	06 FEB 1999 **9902593.4**
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Zeneca Limited 15 Stanhope Gate LONDON W1Y 6LN Great Britain
	Patents ADP number *(if you know it)*	6254007002
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	DRUG COMBINATIONS
5.	Name of your agent *(if you have one)*	DENERLEY, Paul Millington
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	Intellectual Property Department ZENECA Pharmaceuticals Mereside, Alderley Park Macclesfield, Cheshire, SK10 4TG Great Britain
	Patents ADP number *(if you know it)*	1030618002 26863001

		Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

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7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
   a) any applicant named in part 3 is not an inventor, or
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Description	10 / [illegible]
Claim(s)	
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Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Lynda M Slack Date 5th Feb 99

Zeneca Limited Authorised Signatory

12. Name and daytime telephone number of person to contact in the United Kingdom — Lynda M Slack 01625 516173

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DRUG COMBINATIONS

The invention concerns safe non-interacting drug combinations of a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, which is (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid or a pharmaceutically acceptable salt thereof (the Agent) and a drug which is either an inducer, inhibitor or a substrate of P450, in particular P450 isoenzyme 3A4.

The Agent is disclosed in European Patent Application, Publication No. 0521471, and in Bioorganic and Medicinal Chemistry, (1997), 5(2), 437-444 as an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) which is a major rate-limiting enzyme in cholesterol biosynthesis. The Agent is taught as useful in the treatment of hypercholesterolaemia, hyperlipoproteinaemia and atherosclerosis.

Hypercholesterolaemia is one of the strongest risk factors for atherosclerosis which is associated with coronary artery disease (including Angina Pectoris, Myocardial Infarction and Mortality), stroke (including Cerebro Vascular Accident and Transient Ischaemic Attack) and peripheral arterial occlusive disease. Several types of hypercholesterolaemia exist. The magnitude of hypercholesterolaemia may have consequences for the therapy, but in general, any reduction of elevated plasma cholesterol levels results in an improvement of the risk profile. Diet is an essential first step, but the therapeutic potential of drug therapy is significantly larger. Several types of drug therapy for hypercholesterolaemia are currently available. Guidelines exist for the treatment of hypercholesterolaemia, American Heart Association (AHA) (Anon 1988), Updated Sheffield treatment tables (Ramsay 1996) and Recommendations of the task force of the European Society of Cardiology Guidelines (Pyorala 1994).

HMG CoA reductase inhibitors effectively inhibit cholesterol synthesis in the liver through stimulation of the low density lipoprotein (LDL) receptors. These drugs are currently pre-eminent in the treatment of all hypercholesterolaemia, except the relatively rarely occurring homozygous familial hypercholesterolaemia. HMG Co A-reductase inhibitors have been shown to reduce mortality. Various HMG Co A-reductase inhibitors are marketed, and are collectively referred to as 'statins'.

Despite the impressive benefits of statin therapy, less than optimal therapeutic results may be achieved in some subjects, particularly in the more severe classes of hypercholesterolaemia. This can be due to the occurrence of reversible increases in liver transaminase levels at higher dose levels of statins as well as differences in efficacy. Clinically important (>3 times upper limit of normal [ULN]) elevations in serum alanine aminotransferase (ALT) have been reported for atorvastatin in 0.8 per cent of patients (European Summary of Product Characteristics [SmPC] for atorvastatin [Lipitor™]). In all cases the effect is dose-related and reversible.

(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl] (3R,5S)-3,5-dihydroxyhept-6-enoic acid or a pharmaceutically acceptable salt thereof (the Agent) is also a statin and belongs to the class of what is now called in the literature a "super statin". The first generation statins (such as lovastatin, pravastatin and simvastatin - prodrug derivatives of fungal metabolites and fluvastatin) are categorised in that they achieve only a limited cholesterol lowering affect, with often their dose administered limited by elevations in serum ALT. Second generation "super statins" (such as atorvastatin - synthetic compounds structurally distinct from first generation compounds) inhibitors are categorised in that they lower cholesterol levels to a much higher degree than the earlier first generation of statins before their dose is limited by serum ALT levels. The success of the superstatins over the first generation of statins is best evidenced by the success of atorvastatin [lipitor™]. Since its launch in the USA atorvastatin has reached sales in 1998, doubling from 1997, of $2.2 billion, with atorvastatin capturing 38% share of new prescriptions for cholesterol-lowering agents in the US and is now the most widely prescribed hypolipidaemic in the US (Warner-Lambert 1998 annual results).

However the one major disadvantage of the currently available "super statin" , atorvastatin, is that atorvastatin is metabolised by cytochrome P450 enzyme which may cause drug interactions with other drugs which are inducers, inhibitors or substrates of the same P450 enzyme which metabolises atorvastatin. All of the first generation of statins are metabolised by P450 also. However, the rate of metabolism of pravastatin is sufficiently low that it is less clinically relevant to potential drug interactions.

Nearly all drugs are metabolised to some degree in the human generally to a less lipid soluble compound which is more easily excreted by the kidney. Many of the drug metabolic

enzymes are found in the endoplasmic reticulum (which form microsomes upon homogenisation) of hepatocytes. The liver is the major site of drug metabolism because the liver cells (hepatocytes) contain particularly high concentrations of drug metabolising enzymes. Cytochrome P450 is a family of isoenzymes found in hepatic microsomes. Six specific P450 isoenzymes are responsible for the metabolism of most of the commonly used drugs, namely P450 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4.

One adverse event which is found with the use of statins is myopathy, defined as symptoms of muscle pain, tenderness and weakness, with creatinine kinase (CK) values >10 x ULN also reported for statins in general. In severe cases this can lead to rhabdomyolysis. The incidence of raised CK levels (>3x ULN) for statins has been reported as 3.1 per cent. (SmPC for atorvastatin). Myopathy and rhabdomyolysis have been associated with taking a statin in combination with gemfibrazil, niacin, cyclosporin or erythromycin, (HMG CoA reductase inhibitors, Hinninglake 1992) which are all substrates for P450 3A4. These adverse events are probably related to the metabolism of most statins by cytochrome P450 3A4, leading to interactions with drugs which induce, inhibit or are a substrate of this enzyme.

The Agent is not metabolised significantly by cytochrome P450 3A4 and therefore does not possess the same potential for drug interaction shared with other currently available "super statin", i.e. atorvastatin.

Therefore we present as a feature of the invention a non-interacting drug combination comprising a HMG CoA reductase inhibitor, which is the Agent, and a drug which is an inhibitor, inducer or substrate of P450 3A4.

As a further feature of the invention we present use of a HMG CoA reductase inhibitor, which is the Agent, in the preparation of a pharmaceutical composition for use in a non-interacting drug combination therapy with a drug which is an inhibitor, inducer or substrate of P450 3A4.

As a further feature of the invention we present use of a drug which is an inhibitor, inducer or substrate of P450 3A4 in the preparation of a pharmaceutical composition for use in a non-interacting drug combination therapy with a HMG CoA reductase inhibitor, which is the Agent.

By the term "inducer of P450 3A4" we mean a drug which increases the rate at which P450 3A4 metabolises a substrate, for example by increasing the activity of P450 3A4,

decreasing the rate of biological inactivation of P450 3A4 or by increasing the rate of transcription of the P450 3A4 gene.

By the term “inhibitor of P450 3A4” we mean a drug which lowers the rate at which P450 3A4 metabolises a substrate, for example by lowering the activity of P450 3A4 or by lowering the rate of transcription of the P450 3A4 gene.

By the term “substrate of P450 3A4” we mean a drug which is metabolised by P450 3A4.

By the term “combination” we mean either that the Agent and the drug of the combination are administered together in the same pharmaceutical formulation or that the Agent and the drug are administered separately. When administered separately components of the combination may be administered to the patient simultaneously or sequentially.

We have found that the Agent is not metabolised significantly by any of the major cytochrome P450 isoenzymes, namely P450, 1A2, 2C9, 2C19, 2D6 and 3A4. This is a further feature of the invention.

Preferred non-interacting combinations of the invention include those in which the Agent is combined with a drug which is also involved in lowering cholesterol and is also an inducer, inhibitor or substrate of P450 3A4. Examples include fibrates, such as bezafibrate, clofibrate, ciprofibrate, fenofibrate and gemfibrizol (preferably fenofibrate), and niacin.

Preferred non-interacting combinations of the invention include those in which the Agent is combined with a drug which is involved in treating cardiovascular conditions and which is also an inhibitor, inducer or substrate of P450 3A4. Examples include digitoxin, diltiazem, losartan, nifedipine, quinidine, verapamil and warfarin.

Preferred non-interacting combinations of the invention include those in which the Agent is combined with cyclosporin and /or tacrolimus (FK506) and therefore has utility in treating elevated cholesterol levels in patients who are about to, or have recently undergone, a transplantation operation.

Preferred patients in which the combination of the invention is to be administered are those who suffer from myopathy or rhabdomylosis or who have already been found to suffer from myopathy or rhabdomylosis when treated with HMG Co A reductase inhibitor which is metabolised by P450 3A4.

Other features of the invention include the use of 5-80mg of the Agent in combinations described hereinabove. When a dose range of 5 to 80 mg per day is referred to herein for the Agent other particular dosage ranges which are further independent aspects of the invention include (as appropriate) 10 to 80 mg per day, 10 to 60 mg per day, 10 to 40 mg per day, 5 to 40 mg per day, 5 to 20 mg per day, 10 to 20 mg per day, 20 to 60 mg per day, 20 to 40 mg per day and 40 to 60 mg per day. Particular dosages are 5, 10, 20, 40 and 80mg per day. A particularly suitable starting dose of the Agent in the methods referred herein is 5 to 10 mg per day, especially 10 mg per day.

P450 3A4 substrates include; acetominophen, aldrin, aflentanil, amiodorane, astemizole, benzphetamine, budenoside, carbamazepine, cyclophosphamide, cyclosporin, dapsone, digitoxin, ditiazem, diazepam, erthromycin, etoposide, flutamide, hydroxyarginine, ifosphamide, imipramine, lansoprazole, lidocaine, lovatidine, losartan, lovastatin, midrazolam, nifedipine, omeprazole, quinidine, rapamycin, retenoic acid, steroids, tacrolimus, teniposide, theophyline, toremifene, triazolam, troleandomycin, verapamil, warfarin, zatosetron and zonisamide.

P450 3A4 inhibitors include; clotrimazole, ethinylestradiol, gestodene, itraconazole, ketoconazole, miconazole, diltiazem, naringenin, erthromycin, cyclosporin and triacetyloleandomycin.

P450 3A4 inducers include; carbamazepine, dexamethasone, phenobarbital, phenytoin, rifampin, sulfadimidine, sulfinipyrazone and triacetyloleandomycin.

Examples of other P450 inducers, inhibitors or substrates include those mentioned in Drug Metabolism Reviews (1997) Vol 29, Issue 1+2, pages 413-580, Rendic, S. and Di Carlo, F. J. "Human cytochrome P450 enzymes,: A status report summarizing their reactions, substrates, inducers and inhibitors".

Dosages of the Agent may be administered according to the cholesterol lowering effect desired from a range of 5-80 mg per day in any number of unit dosages. Dosages of the drug which is an inducer, inhibitor or substrate of P450 3A4 are those which are advised for each drug, or which is commercially available. Advantageously due to the lack of interaction at the level of P450 3A4 the skilled person may dose the Agent with a drug which is an inducer, inhibitor or substrate of P450 3A4 with out needing to make any adjustments.

The dose ranges and dosages described above are further independent features of the invention.

Preferably the Agent is bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt (illustrated in figure 1).

Pharmaceutical compositions

The following Example illustrates, but is not intended to limit, pharmaceutical dosage forms which are suitable for use in the invention as defined herein:

Capsule	mg
The Agent	5.0
Lactose	42.5
Cornstarch	20.0
Microcrystalline cellulose	32.0
Pregelatinised starch	3.3
Hydrotalcite	1.1
magnesium stearate	1.1

Capsules containing 1, 2.5 or 10mg of the Agent may be obtained similarly using more or less lactose as appropriate, to maintain a total fill weight of 105mg.

Tablet	mg
The Agent	10
Polyvinylpyrrolidone	2.5
Tricalcium phosphate	20
microcrystalline cellulose	47
Mannitol	47
Sodium starch glycollate	3

Experimental

As used hereinbelow ZD4522 is bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt, as illustrated in Figure 1.

The experiment below is used to determine the in vitro metabolic fate of [^{14}C]-ZD4522 in human hepatocytes and, in addition, to determine the specific P450 isozymes involved in [^{14}C]-ZD4522 metabolism. The latter experiment involves an investigation of the effects of P450 selective chemical inhibitors (see Table 1) on the metabolism of [^{14}C]-ZD4522 by human hepatic microsomes.

COMPOUND:	[^{14}C]-ZD4522.
Chemical name:	Bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt
Isomer:	3*R*,5*S*,6*E* Stereoisomer
Molecular weight:	1001.16 (Ca salt)
Formulation ingredients:	The Agent is dissolved in water to produce a solution suitable for addition to the incubates.

TISSUE SOURCE	Human liver, suitable for the preparation of microsomes and hepatocytes, obtained from The International Institute for the Advancement of Medicine (Exton, USA or Leicester, England). Human hepatocytes may, in addition, be obtained from Biowhittaker Ltd.

EXPERIMENTAL PROCEDURES

(1) METABOLISM OF [^{14}C]-ZD4522 BY HUMAN HEPATOCYTES

[^{14}C]-ZD4522 (1 μM or higher concentration if required for analytical sensitivity) was incubated with hepatocytes (approximately 2 million cells/ml) obtained from two human organ donors. Aliquots were removed into ethanol after 0, 30, 60 and 180 minutes of incubation and stored at approximately -20°C until analysed. The metabolic competence of the hepatocytes was confirmed at the time of incubation by examining their ability to metabolise [^{14}C]-ethoxycoumarin (25 μM); aliquots were removed into methanol at the same time points as for the test compound.

Following incubation of [^{14}C]-ZD4522 with hepatocytes, metabolite profiles were generated by High Performance Liquid Chromatography (HPLC). Identification of the major metabolites was achieved by using Mass Spectroscopy (MS) or Nuclear Magnetic Resonance (NMR). The ability of hepatocytes to metabolise [^{14}C]-ethoxycoumarin was confirmed by HPLC.

ASSESSMENT OF DATA

Data generated was assessed with regard to the following:

(1) Assess whether human hepatocytes metabolise [^{14}C]-ZD4522.

(2) Quantitate the amount of each metabolite formed.

(3) Calculate the total rate of disappearance of parent compound from the incubates.

(4) Identify major metabolites if feasible.

(2) ENZYMES INVOLVED IN ZD4522 METABOLISM

[^{14}C]-ZD4522 (at an appropriate concentration) was incubated with human hepatic microsomes in the absence and presence of selective P450 inhibitors (see Table 1). Similar incubations of [^{14}C]-ZD4522 with individual heterologously expressed P450 isoenzymes was also performed. Incubations were terminated by the addition of an appropriate organic solvent. Metabolite profiles of the incubates are generated by HPLC and metabolite identification by MS and/or NMR spectroscopy.

If the extent of metabolism in microsomes is too low to allow satisfactory analysis of enzymes involved, further work can be initiated using whole cell systems which may support longer incubation periods.

Table 1 Selective chemical inhibitors of P450 isozymes

P450 isozyme	Selective inhibitor
1A2	Furafylline
2C9	Sulfaphenazole
2C19	Omeprazole
2D6	Quinidine
3A4	Ketoconazole

ASSESSMENT OF DATA

Data generated during this study was assessed with regard to the following:-

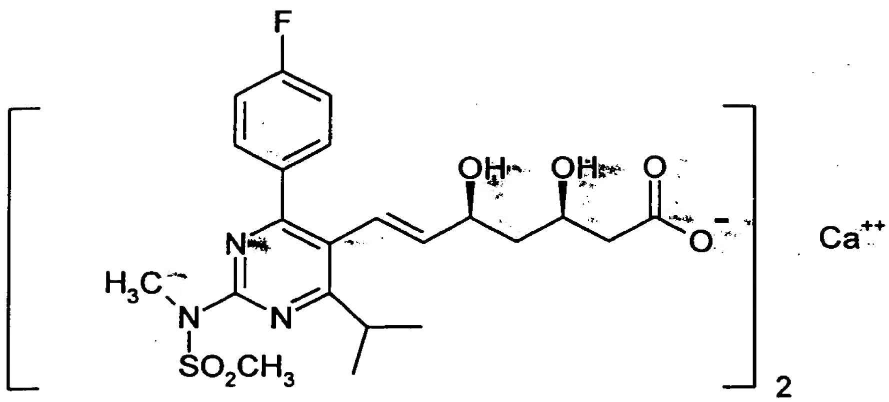
(a) The rate and extent of metabolism of [^{14}C]-ZD4522.

(b) The ability of the selective P450 inhibitors to decrease the metabolism of [^{14}C]-ZD4522 was compared in order to determine the isozyme(s) involved in the metabolism of [^{14}C]-ZD4522.

The ability of individual expressed P450 isoforms to metabolise [^{14}C]-ZD4522 was assessed to aid determination of the P450 isozyme(s) involved in the metabolism of [^{14}C]-ZD4522.

(c) These in vitro data can be used to predict the variability of the pharmacokinetics of ZD4522 in the population and the likely effects on the pharmacokinetics of ZD4522 during co-administration with known enzyme inhibitors/inducers.

It was found that the Agnet was not significantly metabolised by either whole haepatocyts or any of the specific P450 isoenzymes used.



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